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Review

Autophagy, lipophagy and lysosomal lipid storage disorders

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ABSTRACT

Autophagy is a catabolic process with an essential function in the maintenance of cellular and tissue homeostasis. It is primarily recognised for its role in the degradation of dysfunctional proteins and unwanted organelles, however in recent years the range of autophagy substrates has also been extended to lipids. Degradation of lipids via autophagy is termed lipophagy. The ability of autophagy to contribute to the maintenance of lipo-homeostasis becomes particularly relevant in the context of genetic lysosomal storage disorders where perturbations of autophagic flux have been suggested to contribute to the disease aetiology. Here we review recent discoveries of the molecular mechanisms mediating lipid turnover by the autophagy pathways. We further focus on the relevance of autophagy, and specifically lipophagy, to the disease mechanisms. Moreover, autophagy is also discussed as a potential therapeutic target in several key lysosomal storage disorders.

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1. Autophagy and its molecular machinery

The term autophagy was first described in 1966 and is translated from Greek to mean "self-eating" [1]. There are three types of autophagy: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA). Macroautophagy is the most well-studied pathway that involves a multistep process with several vesicular fusion events (Fig. 1). A nascent autophagic vesicle (autophagosome) begins with the formation of an isolation membrane (phagophore) around the cellular component targeted for degradation. The phagophore membrane has been proposed in recent years to originate from several sources including the plasma membrane, endoplasmic reticulum (ER), mitochondria, ER-mitochondria contact sites and the ER-Golgi intermediate compartment [2–8].The phagophore expands and forms a double-membrane structure called autophagosome. Autophagosomes fuse with late endosomes to form intermediate amphisomes, which then fuse with the lysosome to form autolysosomes. It is within the autolysosome that the lysosomal hydrolytic enzymes degrade the autophagic cargo and release the contents into the cytoplasm (Fig. 1). Microautophagy is the process of direct lysosomal engulfment of cytosolic constituents or organelles in a selective or nonselective manner (Reviewed in 9). Lastly, CMA involves the targeting of specific proteins for degradation through the chaperone activity of heat shock cognate 70 (Hsc70) protein [10,11]. Hsc70 is able to recognise the linear peptide sequence KFERQ within substrates and subsequently delivers the protein to the lysosomal lumen via lysosome-associated membrane protein 2a (LAMP2a) [12].

The core genes controlling macroautophagy (for simplicity hereinafter referred to as autophagy) are highly conserved between yeast and mammals. More than 37 autophagy-related (*ATG*) genes have been identified in yeast [13,14]. The formation of a phagophore is a hierarchical process involving two ubiquitin-like conjugation systems, consisting of an E1-like activating enzyme, an E2-like conjugating enzyme and an E3-like ligase. Specifically, in one conjugation system Atg12, a ubiquitin-like protein (UBL) is transferred from the E1-like enzyme Atg7 [15], via an E2-like enzyme Atg10 to form a covalent attachment with Atg5 [16]. The Atg12–5 conjugate forms a complex with Atg16 (in yeast [17]) or Atg16L1 (in mammals [18]) (Fig. 1). The second conjugation system involves a group of UBL proteins from Atg8 (in yeast [19]), or the mammalian Atg8-like family of proteins, comprised of microtubule-associated protein light chain 3 (LC3), as well as Gamma-aminobutyric acid receptor-associated protein (GABARAP) and Golgi-associated ATPase enhancer of 16 kDa (GATE-16) proteins [20]. Using LC3 as an example, first it is modified at the C-terminal by Atg4B to become LC3-I [15,20]. In two subsequent reactions with Atg7 and then Atg3, an E2-like enzyme, the LC3-I is conjugated with phosphatidylethanolamine (PE) to form LC3-II [20] (Fig. 1). The Atg12-Atg5-Atg16L1 complex and LC3-II participate in the formation of phagophores and the initiation of autophagy. As the phagophore is completed to form an autophagosome, the Atg12-Atg5-Atg16L1 complex is removed from the autophagic membrane whereas LC3-II remains attached to the inner membrane (it is removed from the

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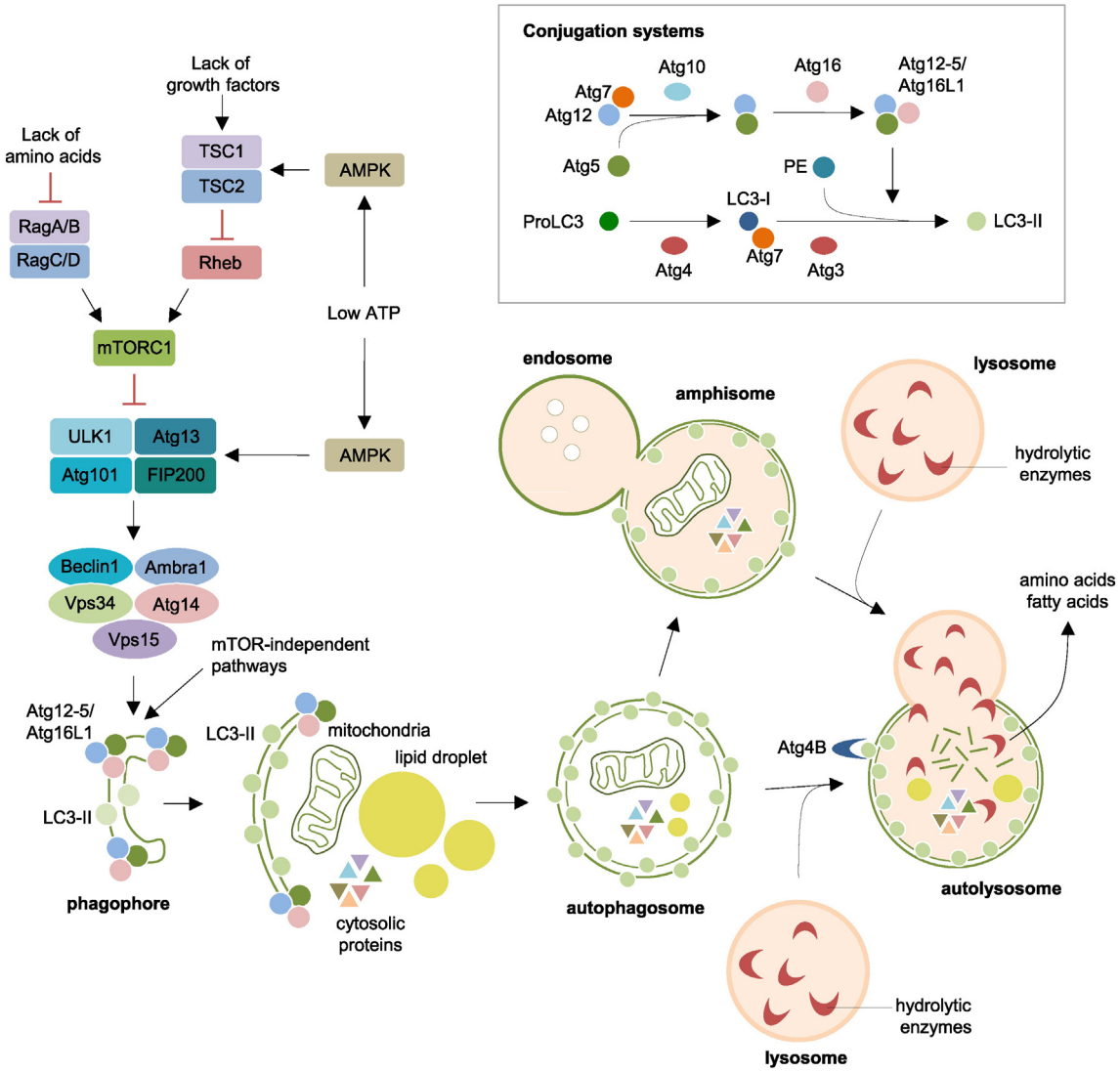

Fig. 1. Regulation of autophagy: Deprivation of growth promoting stimuli (growth factors, amino acids, ATP) activates autophagy via mTORC1 and AMPK signalling pathways which regulate biogenesis of autophagosomes. Macroautophagy can also be activated via mTOR-independent pathways. Conjugation systems (top right insert) promote formation of lipidated membrane-bound LC3-II on nascent autophagosomal membranes which engulf autophagic cargoes leading to the formation of autophagosomes. Fusion events between autophagosomes and endosomes result in the formation of amphisomes which mature into autolysosomes following fusion with lysosomes. Autolysosomes (which may also form by direct fusion of autophagosomes with lysosomes) are digestive organelles where acid hydrolases degrade autophagosomal substrates followed by the efflux of degraded material into cytoplasm.

outer membrane by Atg4B) and is eventually degraded in autolysosomes by lysosomal hydrolases [21] (Fig. 1). There is crosstalk between the two conjugation systems as the Atg12-Atg5-Atg16L1 complex has E3-like ligase activity towards the formation of LC3-II [22]. Knockout of essential autophagy genes, such as *Atg5* or *Atg7*, prevents the formation of phagophores and autophagosomes, and are thus employed to model autophagy-deficient conditions [23,24].

Fusion between late endosomes and autophagosomes to form amphisomes generally precedes the final fusion with lysosomes and the generation of autolysosomes [25–27]. There are several mediators of these vesicle fusion events, such as Rab7, UVRAG, Beclin-1, hVps34, hVps15 and SNARE proteins. Rab7 was identified to play a role in the fusion of late autophagic vacuoles but it was not required for the earlier fusion events between early autophagosomes and endosomes [28]. Beclin-1, Vps34 and Vps15 form a core complex with different roles depending on whether they are bound to Ambra1 and Atg14L, Bif-1 and UVRAG or UVRAG and Rubicon [29–31] (Fig. 1). If the core complex is associated with Ambra1 and Atg14L then autophagosome maturation is promoted [32,33]. UVRAG has roles in promoting autophagosome formation, maturation and endosomal fusion through interactions with the core complex [34]. When Rubicon is bound to the core complex alongside UVRAG, autophagosome and endosome maturation is inhibited [31,32]. In addition, SNARE (SNAP (Soluble NSF Attachment Protein) Receptor) proteins regulate membrane tethering and fusion, events in the autophagic pathway [35]. Recent studies have identified an autophagosomal SNARE, Syntaxin-17 which forms a complex with Atg14 and the SNARE, SNAP-29 to facilitate binding to the late endosomal/lysosomal SNARE, VAMP8 and thus promote autophagosome maturation [36,37]. Interestingly, a non-canonical, alternative autophagy pathway has been described which uses the same basic autophagy machinery, such as ULK1 and Beclin 1, but is Atg5-, Atg7- and LC3-independent [38–40].

Various intracellular signalling pathways act upstream of the autophagic machinery to regulate the autophagy process, these are discussed below.

2. mTOR-dependent regulation of autophagy

The classical regulator of autophagy is the mTORC1 (mammalian or mechanistic Target of Rapamycin Complex 1) pathway, which was first

demonstrated in yeast [41] and later in *Drosophila* [42]. The core mTOR protein exists in two functional, multimeric complexes, mTORC1 and mTORC2, where mTORC2 is generally considered to be important in the regulation of cellular metabolism and the cytoskeleton. mTORC1, on the other hand, functions to integrate a wide range of intra-and extracellular anabolic and catabolic signals to control protein synthesis, metabolism and promote cellular, organ and organismal growth [43–45]. The mTORC1 complex consists of the scaffolding subunit raptor (regulatory associated protein of mTOR), the kinase inhibitors DEPTOR (DEP domain containing mTOR-interacting protein), PRAS40 (proline-rich Akt substrate of 40 kDa), mLST8 (mTOR associated protein) and the protein kinase mTOR [43]. mTORC1 activity is regulated by the availability of intracellular nutrients, energy, oxygen and growth factors; sufficiency promotes mTORC1 activation and phosphorylation of downstream targets including the protein translation regulators, S6K1 (p70-S6 Kinase 1) and the eukaryotic initiation factor 4E (eIF4E) binding protein 1 (4E-BP1) [43], while at the same time inhibiting autophagy. Deprivation of any mTORC1-promoting signals leads to the activation of autophagy and inhibition of anabolic processes [43,46]. Autophagy is induced during energy or nutrient stress to recycle intracellular components, restore the deficiency and promote cellular survival.

2.1. Regulation of autophagy downstream of mTORC1

Several mechanisms by which mTORC1 regulates autophagy have been described. Firstly, under nutrient-rich conditions, mTORC1 directly interacts with the ULK1-Atg13-FIP200 complex and phosphorylates ULK1 and Atg13 which inhibits autophagy. Upon inactivation of mTORC1, ULK1 and Atg13 are dephosphorylated which relieves inhibition of autophagy [47–49]. Secondly, mTORC1 phosphorylates and thereby inactivates DAP1 (death-associated protein 1) and it was suggested to act as a braking system for autophagy [50]. Thirdly, mTORC1 phosphorylates TFEB (transcription factor EB), which prevents its translocation to the nucleus and inhibits the transcription of autophagy and lysosomal related genes [51]. Finally, an mTORC1-dependent post-transcriptional regulatory pathway of autophagy via Dcp2 was identified. In nutrient-rich conditions, DDX6 (a RCK family member) recruits many *ATG* mRNA transcripts to the Dcp2 decapping complex, leading to mRNA degradation and autophagy inhibition, whilst starvation-dependent dephosphorylation of Dcp2 reverses the process [52]. These pathways combined orchestrate autophagy signals downstream of mTORC1.

2.2. Regulation of autophagy upstream of mTORC1

A prerequisite signal for mTORC1 activity is the availability of amino acids which promote the translocation of the complex to the cytoplasmic surface of lysosomes, thus bringing it into close proximity with its activator, the small GTPase, Rheb [53,54]. How amino acids activate mTORC1 is not fully understood but amino acids have been shown to signal via a heterodimeric complex of Rag GTPases, whereby the functionally redundant RagA or RagB forms a complex with RagC or RagD [55] (Fig. 1). The amino acid-dependent nucleotide loading of RagA/B with GTP and RagC/D with GDP promotes the activity of mTORC1 [56]. This nucleotide loading is controlled by a number of regulatory protein complexes [54,57].

Another mechanism of amino acid sensing is mediated by the TSC complex, which consists of three subunits, TSC1, TBC1D7 and TSC2 and which is also regulated by other inputs, including growth factors, via PI3K and Akt [58–60]. The TSC2 subunit of the complex acts as a GTPase activating protein (GAP) for Rheb, promoting the hydrolysis of GTP to GDP and thereby inhibiting its ability to activate mTORC1 [61]. Starvation has also been shown to promote lysosomal recruitment of TSC complex and [62] (Fig. 1). In addition to amino acids and growth factors, mTORC1 activity can be regulated by the cellular energy state through the AMP-dependent kinase (AMPK) which is activated by low levels of ATP in the cell and regulates autophagy via TSC complex [63] and via ULK1 complex which promotes autophagosome biogenesis [64] (Fig. 1).

mTORC1 is an appealing pharmacological target to manipulate autophagy which encouraged great efforts to develop better and more specific inhibitors [65]. Rapamycin is the best known mTOR inhibitor [66], but many rapamycin analogues (rapalogs) have been developed and are in clinical trials.

3. mTORC1-independent regulation of autophagy

In addition to the canonical mTORC1-dependent regulation of autophagy, several mTOR-independent pathways have been described. The main mTORC1-independent mechanisms are mediated by intracellular inositol [67], calcium [68,69], cAMP [68] and the JNK1/Beclin1/PI2KC3 signalling pathway; reviewed elsewhere [70]. Type III PI3 kinase is an important regulator of autophagosome biogenesis and several mTOR-independent signalling cascades, including MAPK-ERK1/2, Stat2, Akt/Foxo3 and CXCR4/GPCR, converge into this PI3K signalling node [71].

Several small molecules that induce autophagy have been described, although their mechanism of action is not always known. For example, trehalose efficiently clears autophagic cargo in an autophagy-dependent, but mTORC1-independent manner [72]. Also, a comprehensive screen of small molecules identified many mTOR-independent small molecule enhancers (SMERs) of autophagy [73]. These and other mTORC1-independent autophagy enhancers have potential therapeutic applications in diseases with perturbation of lipid or protein homeostasis, such as neurodegenerative and lipid storage disorders; reviewed elsewhere [27,70,74,75].

4. An introduction to lipophagy

Despite the classical view that autophagy is a largely bulk, nonselective process, it is being increasingly recognised that there is in fact a remarkable selectivity in the nature of cargo degraded. For instance, through unique organelle-specific adaptors the autophagy pathway is capable of sequestering damaged or aged organelles, oxidised proteins, and even portions for the cytosol, which are then degraded in lysosomes. Accordingly, the selective degradation of endoplasmic reticulum, mitochondria, ribosomes, and peroxisomes are referred to as ERphagy [76], mitophagy [77], ribophagy [78] and pexophagy [79], respectively.

We have previously shown that cellular lipid stores are also targeted for lysosomal degradation via a process termed “lipophagy” [80]. The identification of lipophagy as a new process dedicated to cellular lipid removal has mapped autophagy as an emerging player in cellular lipid metabolism [81]. Indeed, a number of studies have now demonstrated roles for autophagy in lipid droplet (LD) turnover in cells as diverse as hepatocytes [80,82], hypothalamic [83] and striatal neurons [84], glial cells [84], macrophage foam cells [85], enterocytes [86], T cells [87], fibroblasts, adipocytes and adipose-resident macrophages [88], prostate carcinoma cells [89], as well as in *Saccharomyces cerevisiae* [90], *Caenorhabditis elegans* [91], certain fungal species [92], and in staple crop such as rice [93]. It is likely that activation of lipophagy in each of these cell types is context-specific and coupled to energetic requirements to perform a certain function. For instance, lipophagy is acutely activated in livers during fasting to rapidly degrade the large lipid bolus delivered from the adipose tissue [80]. On the other hand, hypothalamic neurons employ lipophagy as a means to generate free fatty acids that boost levels of agouti-related peptide (AgRP) [83], a neuropeptide that stimulates feeding by activating second-order neurons in the hypothalamus. Similarly, lymphocytes require lipophagy to generate the energy necessary for their activation in response to antigenic challenges [87]. Although the upstream signals activating lipophagy

could be cell and context-dependent, it is likely that the core components and the mechanism(s) of lipophagy activation are conserved in most cell types. Thus, the ubiquitous nature of lipophagy is a testament to the fact that cells, tissues, and species have evolved with a mechanism in place to counteract excessive lipid build-up or to rapidly utilise lipid reserves for specialised functions.

All cells store lipids during times of nutrient sufficiency in the form of lipid droplets (LDs). When nutrients are scarce, cells rapidly deplete their energy reserves, including LDs, in order to meet their basic energetic needs. Physiological fat storage occurs in cytoplasmic LDs. An LD is, in essence, an organelle consisting of a neutral lipid core of triglycerides and cholesteryl esters that is limited by a phospholipid monolayer and a family of unique LD coat proteins, now classified as perilipins (PLINs) [94]. Each LD ranges from 0.1 to 10 μm in size, however cells that specialise in fat storage, for instance adipocytes, may have LDs that are 10–100 times larger. It is well-established that neutral lipases, adipose triglyceride lipase (ATGL) [95], hormone-sensitive lipase (HSL) [96] and monoacylglycerol lipase (MGL) [97] act in tandem to rapidly mobilise fat droplets during nutrient deprivation. During lipolysis, which is best characterised in the adipocyte, activation of protein kinase A leads to the phosphorylation and proteasomal degradation of perilipin 1 (PLIN1) [98]. This results in the release of comparative gene identification-58 (CGI-58) which then activates ATGL and initiates triglyceride breakdown [99], as discussed in subsequent sections. Together, this process allows lipases to dock upon and consume the exposed LD core. Conversely, G0/G1 switch 2 (G0S2) protein inhibits ATGL [100]. Since starvation leads to activation of lipolysis and autophagy, we envisioned a possible role for autophagy in the turnover of LDs.

In cultured hepatocytes and mouse embryonic fibroblasts, chemical and/or genetic inhibition of autophagy resulted in increased LD number and size [80]. Conversely, activation of autophagy by rapamycin increased the colocalisation of the LD marker, BODIPY with the lysosomal marker (LAMP1) indicating activation of lipophagy. Furthermore, autophagosome and lysosome fractions from fasted mice both contained LD-associated PLINs, and liver-isolated LDs co-purified with autophagosome marker LC3-II [80]. Moreover, liver-specific *Atg7* knockout mice displayed large livers that were accumulating cellular debris, including damaged organelles, ubiquitinated proteins, and a significant increase in lipid content [80]. Histological analyses revealed that livers lacking *Atg7* closely resembled those observed in cases of human non-alcoholic fatty liver disease, thus underscoring a critical role of this pathway in hepatic lipo-homeostasis. Together, these results demonstrate a fundamental role for autophagy in cellular lipid utilisation [80].

4.1. Mechanisms of lipophagy

The mechanisms regulating lipophagy, in particular those related to how autophagy selectively identifies and sequesters LD, remain unknown. While the search for the elusive lipophagy adaptor continues, it has been suggested that lipophagy entails complete or "piecemeal" consumption of LD by autophagosomes [80] (Fig. 2). In the course of our studies, we observed that isolated LDs from fasted mice displayed enrichment of both cytosolic LC3-I and autophagosome-bound LC3-II which suggested that the conversion of LC3-I into LC3-II occurs at the surface of LDs [80]. Indeed, our unpublished *in vivo* work, and recently published *in vitro* work from the Cuervo group [101] indicate that, in fact, several regulatory autophagy proteins are enriched in LDs. This would support the idea that *de novo* biogenesis of autophagosomes to sequester LDs occurs at the LD surface. It is interesting to note at this point that the lipase, PNPLA5 has been shown to be required for efficient autophagy of diverse form of substrates [102]. In a more recent study, Shpilka et al. have identified that in yeast, enzymes required for synthesis of triglycerides (Dga1 and Lro1) or steryl esters (Are1 and Are2), as well as the lipase Ldh1 are required for autophagy [103]. These studies indicate that active lipid metabolism at the LD surface or at LD-ER contact sites provide the metabolic energy required for the *de novo* formation of autophagosomes [102,103].

Rambold et al. recently demonstrated that acutely starved cells use LDs to supply mitochondria with fatty acids for β-oxidation (break down of fatty acids to generate acetyl-CoA). Interestingly lipase activity was necessary for fatty acid delivery from LDs into mitochondria [104]. Bulk autophagy, but not lipophagy was shown to be involved in shuttling cellular membrane-derived fatty acids into the cytoplasm and their association with LDs, which was required for mitochondrial oxidative metabolism [104]. In our view, it would appear that locally generated free fatty acids made available by lipases and rapidly oxidised in the mitochondria are likely to sustain an active feed-forward mechanism to maximise LD breakdown through *de novo* autophagosome formation. Indeed, it was suggested that LDs could be mobilised into phospholipids necessary for autophagosomal membrane formation and growth whilst PNPLA5, a neutral lipase that localises to lipid droplets, was needed for optimal initiation of autophagy [102].

Given these complex cellular dynamics, it is not surprising to note that the cellular components that generate the membranes to form autophagosomes, e.g., ER or mitochondria [6] are the very substrates that are eventually devoured by the autophagic machinery.

Regarding the mechanistic basis for the regulation of lipophagy, an important question is how does the autophagic machinery recognise LDs as a substrate? While polyubiquitin chains of specific lysine linkages are a well-established coding system to distinguish proteins and organelles intended for degradation, it remains unclear whether polyubiquitination could also serve to tag and degrade LDs. On that note, ancient ubiquitous protein (AUP1) is a protein that has been shown to localise to LDs and interact with an E2 ubiquitin-conjugating enzyme, Ube2g2. It is possible that the AUP1-Ube2g2 complex tags LD components for degradation [105]. However, since it is well known that LDs serve as a cellular buffer that sequesters and inactivates critical proteins, for instance a subset of histone proteins are sequestered by LDs during development [106], it would require further work to determine whether AUP1-Ube2g2 complex indeed labels LDs for degradation. The second relevant question here is whether crosstalk between different proteolytic systems regulates turnover of LD components and whether lipophagy is involved? A study using Chinese hamster ovarian cells has shown that PLINs are stabilised and prevented from degradation when cells are treated with fatty acids [107]. By contrast, PLINs were observed to be rapidly degraded when triglyceride biogenesis was blocked [107]. In addition, PLINs were found to be polyubiquitinated and selective proteasomal inhibitors blocked PLIN degradation, indicating that the proteasome participates in their turnover [107].

4.2. Role of CMA in lipophagy

Interestingly, recent work from the Cuervo group has identified that chaperone-mediated autophagy (CMA), may also play a significant role in selective degradation of PLINs such as PLIN1 exclusively expressed in adipocytes and PLIN2 and PLIN3 expressed ubiquitously [101] (Fig. 2). Lamp2A knockout mice showed pronounced hepatic steatosis, coupled with insulin resistance [108]. The fact that LDs accumulate in a CMA-deficient model is intriguing since 1) macro- and micro-autophagy are functional in this model, and in fact LAMP-2A KO mice display a compensatory upregulation of macroautophagy *in vitro* and *in vivo*, and 2) only proteins and not lipids can be CMA substrates. This led to their hypothesis that CMA is required to eliminate LD coat proteins, e.g., PLINs as a prerequisite for lipolysis to occur. The authors show unequivocally, that PLIN2 and PLIN3 are CMA substrates and that their degradation increases in conditions of increased lipolysis, e.g., during starvation [101]. Since PLINs are gatekeepers of LD mobilisation, the inability to degrade them in the CMA-deficient model systems, results in LD accumulation and

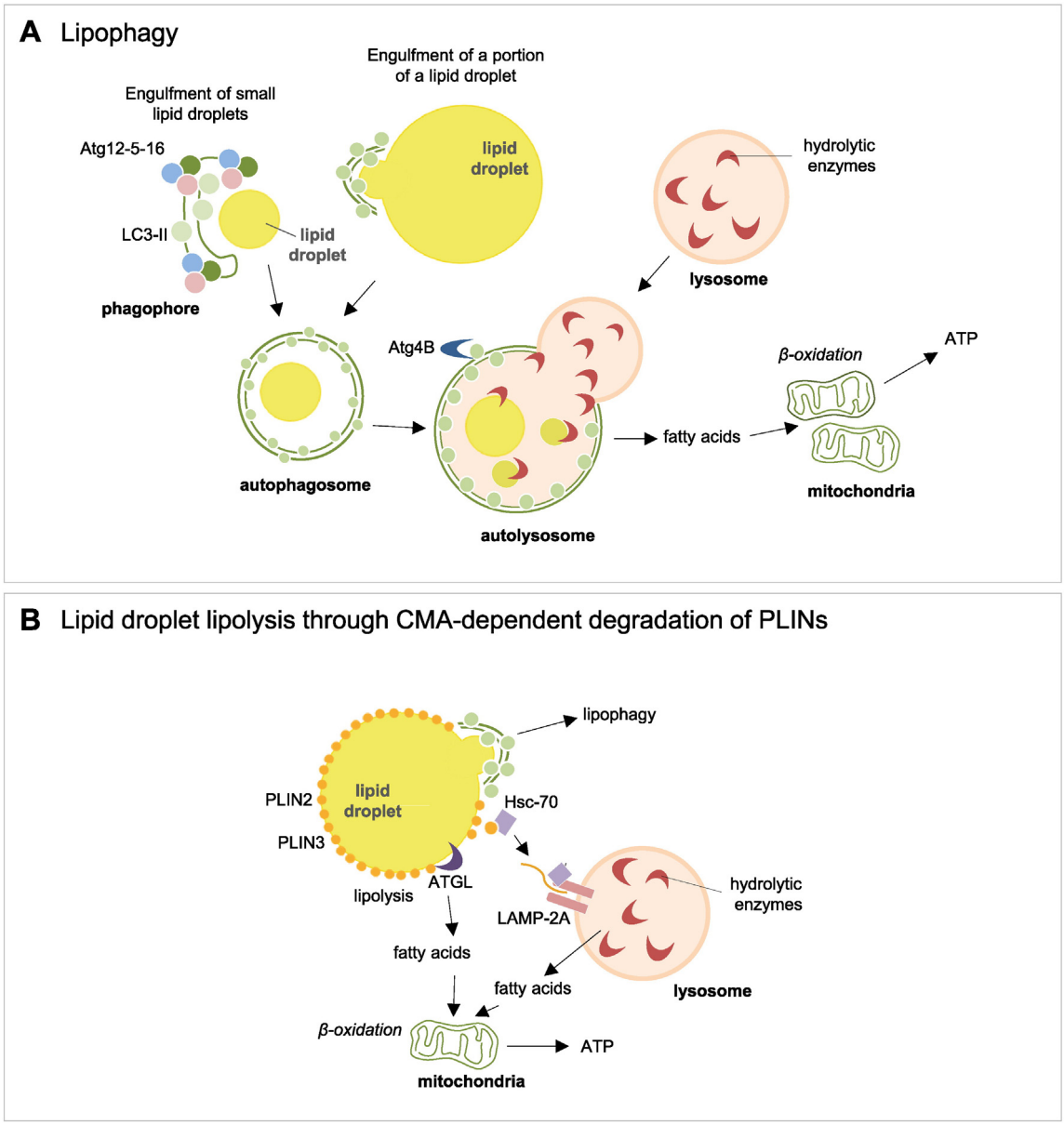

Fig. 2. Autophagy degrades lipid droplets: A — LC3-II positive membranes engulf small lipid droplets or sequester portions of large lipid droplets. Autophagosomes deliver the lipid cargo to lysosomes wherein acid hydrolases degrade lipids. Fatty acids are released into the cytosol and these undergo mitochondrial β-oxidation for provision of energy. B — Activation of chaperone-mediated autophagy degrades the lipid droplet coat proteins PLIN2 and PLIN3 through coordinated action of Hsc70 and the LAMP-2A receptor. Consequently, the lipid droplet surface becomes accessible to cytosolic lipases, e.g., ATGL that hydrolyzes the lipid droplet resident triglycerides to generate free fatty acids, and the autophagic machinery proteins.

steatosis. The authors show that removal of PLINs from the LD surface occurs prior to the docking of autophagy proteins and cytosolic lipases [101] (Fig. 2). *In vitro* and *in vivo* studies, including live cell video-microscopy, show decreased association of autophagy proteins and LAMP1 with LDs in the CMA-null cells. Moreover, over-expressing a PLIN2 mutant lacking a functional CMA-targeting motif, KFERQ, in wild-type cells was sufficient to prevent its degradation and block the association of cytosolic lipases and autophagy effector proteins with LDs [101]. Therefore, functional CMA is essential for removal of LD proteins from specific areas of the LDs, which in turn 'primes' these regions for the recruitment of the lipolytic machinery – lipases and autophagy proteins. The authors further go on to suggest that phosphorylation of PLIN2 is important for its recognition and degradation via CMA opening thus a new area of exploration in the understanding of the first trigger for LD turnover.

4.3. Receptor proteins in lipophagy

While CMA and the proteasome degrade LD coat proteins, it could also be possible that selective autophagy *per se* contributes to elimination of LD proteins. Over the last few years, a number of distinct selective autophagy receptors have been identified, e.g., SQSTM1/p62 [109], NDP52 [110], optineurin [111], and NBR1 [112] to mention a few, and it is quite possible that any of these could also serve as the LD receptors. Finally, another protein of interest that could serve as a possible cargo recognition receptor is Huntingtin. Huntingtin was recently shown to act as a scaffold for selective autophagy [113] and mutations in Huntingtin have been shown to lead to the generation of large, empty autophagosomes that fail to sequester cargo [114]. Since cells expressing mutant Huntingtin have revealed remarkable lipid accumulation [114], it could be possible that Huntingtin is a LD recognition receptor protein. Finally, an intriguing possibility is that LC3 can recognise and

bind phospholipids directly as has been proposed for LC3-cardiolipin interaction during mitophagy [115]. Further studies will be necessary to validate these hypotheses and uncover the molecular mechanisms of how the autophagic apparatus degrades LDs.

5. Lipophagy in fatty liver disease

Acute fatty acid exposure results in the activation of autophagy in cultured hepatocytes [80] and in neurons [83]. This autophagy activation likely serves to eliminate LDs generated from the rapid influx of lipids into the cell. In contrast, prolonged lipid exposure, e.g. when mice are fed a high fat diet, results in the suppression of autophagy and lipophagy as noted by decreased LD-associated LC3 and decreased areas of degradation in LD [80]. It has also been reported that levels of the E1-like ligase, Atg7 are diminished in high fat diet-fed mice and in the leptin-deficient *ob*/*ob* mouse model [82]. This reduction may contribute, at least in part, to the autophagy suppression observed during chronic over-nutrition. In addition to depletion of Atg7 protein levels, a number of factors may contribute to suppression of autophagy. For instance, obesity is associated with hyper-activation of mTORC1 signalling which is a well-established inhibitor of autophagy. On the other hand, it has been shown that a prolonged high fat diet not only blocks the fusion of autophagosomes and lysosomes [116], but inhibits CMA [117]. Given the recent elucidation of the selective role for CMA in the degradation of LD proteins, it is conceivable that blockage of autophagy and CMA could bear a strong inhibitory effect on LD breakdown by lipophagy. Given these bearings, it is quite likely that suppression of autophagy and lipophagy following chronic over-nutrition will set up a vicious cycle that will, in turn, promote lipid accumulation and metabolic compromise.

Accumulation of fat begets inflammation, and Yang *et al.* have shown that high fat-induced autophagy suppression is associated with the development of hepatic inflammation and endoplasmic reticulum stress [82], while in contrast, liver-specific overexpression of Atg7 restores autophagy and ameliorates hepatic steatosis in these models [82]. Similarly, other studies have revealed that hepatocytes deficient in autophagy are susceptible to cell death from oxidant stress [118], and that lipophagy, in fact, ordinarily provides the fatty acid substrates that are oxidised to generate the energy and prevent cell death [118]. In addition, fat-laden and inflamed livers produce tumour necrosis factor (TNF)-α and recent work from the Czaja laboratory has shown that inhibiting autophagy predisposes livers to severe hepatotoxicity from TNF-α and galactosamine [119]. Furthermore, overexpressing an essential component of the Class III PI3K complex, Beclin1, prevents hepatotoxicity in response to tumour necrosis factor [119]. In addition to these mechanisms, it cannot be excluded that lipophagy detoxifies the liver by eliminating cytotoxic lipid species such as sphingolipids and ceramide generated during obesity. Thus, defective autophagy in the background of hepatic steatosis could be the “second hit” in the proposed “two-hit theory” that defines the pathobiology of a normal liver becoming steatotic and then progressing to non-alcoholic steatohepatitis [120]. These results also provide proof of concept that activating autophagy, and specifically lipophagy, could be a novel strategy against obesity-associated fatty liver disease and development of steatohepatitis. Underscoring the necessity of lipophagy in maintaining hepatic lipo-homeostasis is the fact that livers remain vulnerable to steatosis due to their central role in handling lipid flux, and since lipases ATGL and HSL, which control lipolysis in adipose tissue, are poorly expressed in the liver [95,121]. In this respect, it has been shown that in cells that are lacking ATGL and HSL, lipophagy is not induced, but the lack of free fatty acids for energy production can be compensated for by upregulation of autophagy [122]. Whether activating autophagy and specifically lipophagy is a viable therapeutic option in treating human fatty liver disease it remains to be seen in the years to come.

6. Lysosomal lipid storage diseases

Lysosomal lipid storage disorders are a group of rare inherited diseases that cause accumulation of lipids in the lysosomes of cells that leads to cellular toxicity [123,124]. Neurons are particularly sensitive to lipid accumulation and therefore patients normally exhibit neurodegeneration often with stunted brain development. Many of these diseases are fatal at a young age and treatment options are limited. Autophagy has been identified as a major pathway for the metabolism of lipids in cells [80]. Perturbations in autophagy, or specifically lipophagy, could thus be linked to the accumulation of cellular lipids in patients with lipid storage disorders. Although there is growing evidence for the role of autophagy in lipid storage disorders (see Table 1), further investigation is required to fully understand the mechanisms and investigate the therapeutic potential of targeting autophagy in these diseases.

6.1. GM1 gangliosidosis

GM1 gangliosidosis is a rare lysosomal storage disorder that clinically exists in three forms based on the age of onset. Type I (early infantile form) occurs before 6 months of age with a high risk of death. It is characterised by psychomotor regression, central nervous system defects and musculo-skeletal abnormalities [125,126]. Type II (late infantile or juvenile form) presents between 7 months and 3 years of age and is associated with ataxia, dwarfism and neurodegeneration [127]. Type III (adult form) is least symptomatic, and can occur anywhere between 3 and 30 years with muscle malfunction [127,128].

GM1 gangliosidosis is caused by mutations in the *GLB1* gene, which leads to deficiency in the activity of lysosomal β-galactosidase [129] and an accumulation of lipids such as GM1 ganglioside. The nervous system of patients is the most severely affected area [130–132]. Although it is not entirely clear whether perturbation in autophagy is underlying clinical features of GM1 gangliosidosis, accumulation of autophagosomes (LC3-II) was found in the cortex and hippocampus in a mouse model of GM1 gangliosisosis (β-galactosidase deficient $\beta Gal^{-/-}$ mice) [133,134]. Despite the fact that autophagic flux data were not reported in this study, the elevation in LC3-II levels was shown to be independent of mTOR activity [134]. This is possibly indicative of impairment in autophagic flux arising due to inhibition of autophagosome maturation rather than increased synthesis.

Presently, there are only symptomatic treatments for the disease with no effective cure. However, there have been some promising results from *in vitro* studies and animal models. Addition of the chemical chaperone N-octyl-4-epi-β-valienamine (NOEV) to cultured human and mouse fibroblasts rescued the disease phenotypes [135]. In addition, when NOEV was administered to mice that express the mutated human β-galactosidase that causes Type II GM1 gangliosidosis it decreased GM1 accumulation in the cerebral cortex and brainstem [135]. Another potential therapeutic treatment option being explored is the use of adenoviral associated virus gene delivery of *βGal*, which improved lysosomal storage clearance in nervous tissue and increased lifespan in $\beta Gal^{-/-}$ mice [136].

6.2. Fabry disease

Fabry disease (FD) is a rare, inherited, metabolic disorder with clinical manifestations including lipid accumulation in the cornea [137], heart defects, angina and exercise intolerance [138–141]. It is more common in males than females, and is caused by mutations in the gene encoding α-galactosidase A located on the X-chromosome [142–145]. Without functional α-galactosidase A, cells accumulate globotriaosylceramide and other glycosphingolipids in various tissues including the kidney [123,124]. This is associated with defective autophagy since increased levels of LC3 and p62, and accumulation of vacuoles, where found in renal cells from FD patients compared to the

Table 1
Disease related mutations and their effect on the autophagy pathway.

Disease	Gene	Protein	Function	Lysosomal accumulation	Deregulation in autophagy	Reference
Fabry disease	GLA	alpha-galactosidase A	Homodimeric glycoprotein, hydrolyses glycolipids and glycoproteins	Failure to catabolise alpha-D-galactosyl glycolipid moieties	Inhibition caused by disruption of autophagy-lysosome pathway	[142], [146]
Gaucher disease	GBA	glucosidase, beta, acid	Lysosomal membrane protein, Involved in glycolipid metabolism	Accumulation of glucocerebrosides	Block in autophagic flux	[154], [160]
Glycogenoses	GAA	acid alpha-glucosidase	Lysosomal enzyme, converts glycogen to glucose	Glycogen accumulation	Accumulation of autophagosomes	[174], [176]
GM1 Gangliosidosis	GLB1	β-galactosidase	Lysosomal enzyme, hydrolyzes beta-galactose from ganglioside substrates	Accumulation of GM1 gangliosides	Accumulation of autophagosomes	[128], [134]
Mucopolysaccharidoses	More than 10 genes identified		Lysosomal enzymes	Glycosaminoglycans	Defective autophagosome-lysosome fusion	[178]
Mucolipidoses						
ML IV (Gangliosidose)	MCOLN1	Mucolipin 1	Cationen channel receptor protein, regulation of lysosomal exocytosis	Deficiency of transport channel receptor protein	Impairment of autophagy	[227]
Neuronal Ceroid-Lipofuscinoses						
	CLN3	Battenin	Role in pH homeostasis and Catepsin D function	Accumulation of ceroid lipofuscin	Disruption of autophagy vacuole maturation and impaired mitophagy	[239], [241]
	CLN6	non-glycosylated endoplasmic reticulum (ER)-resident membrane protein	Likely to be involved in the degradation of post-translationally modified proteins	Protein accumulation	Accumulation of autophagic vacuoles	[243]
Niemann–Pick disease						
Type A and B	SMPD1	ASM (Acid sphingomyelinase)	Enzyme, conversion of sphingomyelin to ceramide	Accumulation of sphingomyelin	Inefficient autophago-lysosomal clearance	[188], [200]
Type C	NPC1 (95%) NPC2 (5%)	NPC1, NPC2	Cholesterol export from late endosomal/lysosomal compartment	Accumulation of cholesterol	Defective amphisome formation	[187], [196], [195]

control cells [146]. Likewise, increased immunoreactivity for LC3 and LAMP-1 along with aberrant accumulation of phosphorylated α-synuclein was found in the brain sections of α-galactosidase A-deficient mice [147]. Additionally, knockdown of α-galactosidase A by lentiviral shRNA in human renal cells increased LC3-II levels but was associated with downregulation of mTOR and AKT activity [148]. Although this study may be indicative of an induction of autophagy, accumulation of autophagic substrates reported in other studies with patient cells points towards impairment in autophagic flux; however, mechanistic details are yet to be addressed.

Interestingly, enzyme replacement therapy (ERT) in patients for 3 years with the drug, agalsidase alfa, reduced the staining intensity of LC3-II and p62, and the vacuolar phenotype, indicating that the defect in autophagic flux could possibly be restored after introduction of the functional human α-galactosidase A [146]. Several treatment options have been employed to treat FD in the last few decades. ERT was tested in two patients using healthy donor plasma which contained the missing enzyme [149]. Two recombinant versions were used in a 30–36 month trial which resulted in a reduction of globotriaosylceramide concentration in the blood. [150]. Since 2001 agalsidase alfa has been available for treatment in patients [151]. Production of recombinant enzymes is prohibitively expensive and its cost effectiveness compared to the benefits to the patients is debatable [152]. Additional investigation into a role for autophagy in FD is warranted, as chemical modulation would be more economically viable than ERT.

6.3. Gaucher's disease

Gaucher's disease (GD) is the most common inherited lysosomal storage disorder. The disease symptoms include hepatomegaly, splenomegaly, haematological disorders, skeletal weakening and conjunctival degeneration [153]. There are three clinical subtypes: type I (non-neuropathic, adult form), type II (acute neuropathic, infantile form) and type III (chronic neuropathic, juvenile form). Mutations in the gene encoding glucosylceramidase (also known as glucocerebrosidase) that diminish or eliminate the activity of this enzyme is the underlying cause of GD [154]. Deficiency in glucosylceramidase causes the accumulation of glucosylceramide in cells that can lead to cytotoxicity [155]. Deficiency of another lysosomal protein, Saponin C, which is an enhancer of glycosphingolipid hydrolase activity of glucosylceramidase, leads to a variant form of GD with accumulation of glucosylceramide in the macrophages and central nervous system. In types II and III, the disease pathology displays accumulation of lipids, inclusions and cell death in neurons, which has been recapitulated in transgenic models *in vivo*, such as in $Gba^{flox/flox}$; *nestin-Cre* [156] and $PSAP^{-/-}$ (gene which encodes for precursor of Saponin C) mice [157].

Studies in mouse models of GD indicate there may be defects in autophagy. Accumulation of ubiquitinated protein aggregates, insoluble α-synuclein, lysosomes as well as autophagic substrates such as p62 occurs in the brain of transgenic mice with *glucosylceramidase* or *PSAP* deficiency [157–159]. In addition, accumulation of dysfunctional mitochondria due to defective mitophagy was found in neurons and astrocytes in a mouse model of GD ($gba^{-/-}$) [158]. Likewise, impaired degradation of autophagosomes was seen in Saponin C-deficient patient fibroblasts. This was suggested to arise due to a block in autophagic flux caused by diminished activity of the lysosomal enzymes, cathepsins B and D [160]. In this system, over-expression of functional lysosomal hydrolases restored the degradative capability of the autolysosomes [160]. A recent study has demonstrated that a block in autophagic flux arising due to impaired autophagosome maturation in neuronal cells derived from GD patient-specific induced pluripotent stem cells (iPSCs) [161]. Furthermore, downregulation and reduced stability of the transcription factor EB (TFEB; the master regulator of lysosomal genes [162]), as well as a reduction in lysosomal gene expression, was found in GD iPSC-derived neurons. In this study, treatment of mutant neuronal cells with recombinant glucocerebrosidase abrogated the lysosomal dysfunction and autophagy block; an effect enhanced by overexpression of TFEB but not with TFEB alone, without the recombinant enzyme [161].

ERT has been developed for the treatment of GD [163]. There are three enzyme replacement drugs: Imiglucerase [164], Velaglucerase alfa [165] and Taliglucerase alfa [166]. All these drugs are recombinant versions of glucocerebrosidase that have slightly different pharmacological properties [167,168]. Another option for treatment is substrate reduction therapy, such as with Eliglustat tartrate that functions by blocking the activity of glucosylceramide synthase, the enzyme which catalyses the production of glucosylceramide [168,169]. This drug was shown to be safe for human intake in phase 1 clinical trials [170] and was further demonstrated to reduce the levels of ganglioside GM3 and glucosylceramide in phase 2 trials [171,172]. However, ERT is extremely expensive and thus justifies the search for other therapeutic targets [173]. Although the potential for autophagy modulation has not been investigated rigorously, a recent study in GD iPSC model has reported neurotoxicity caused by treatment with rapamycin [161].

6.4. Glycogenoses

Glycogenoses are a group of diseases caused by defective metabolism or degradation of glycogen which results in its accumulation within enlarged lysosomes. Glycogenoses disorders include Pompe disease, Von Gierke disease and Her's disease among others. Pompe disease is caused by a deficiency or absence of the lysosomal enzyme, acid alpha-glucosidase (GAA) which prevents glycogen conversion to glucose. ERT has had therapeutic success in treating the cardiac defects characteristic of Pompe disease however other disease symptoms persist in the other major site of clinical manifestation, skeletal muscle tissue (reviewed in 174). It has been postulated that disease persists as a result of the general defect in membrane trafficking seen within Pompe models and patients. As a result, there is an accumulation of autophagosomes (as a result of significantly reduced lysosomal fusion [175] and associated p62 and ubiquitin inclusions all of which contribute to cellular toxicity [161,176]. Furthermore, a build-up of lipofuscin within accumulated vesicles and in the cytoplasm further perturbs membrane trafficking and mitochondrial turnover which may cause defective redox balance in the cells [174]. The resulting accumulation of cellular contents causes cell toxicity and perturbs muscle cell integrity, contractile function and survival and leads to the muscle weakening observed in Pompe disease mouse models and patients. Inhibition of autophagy is currently being explored as a potential therapeutic intervention in Pompe disease. Skeletal muscle-specific knock-out of *Atg5* and *Atg7* leads to reduced glycogen delivery and therefore accumulation in the lysosome (instead it is metabolised safely in the cytoplasm) and increased lysosomal delivery of ERT (which is normally perturbed in muscle because of the defects in membrane trafficking to lysosomes) [161,177]. These studies suggest that targeting autophagy may provide benefits to Pombe disease aetiology.

6.5. Mucopolysaccharidoses

Mucopolysaccharidoses and the related, multiple sulfatase deficiency (MSD) are group of diseases characterised by defective degradation of glycosaminoglycans (GAGs) and sulphatases, respectively, both of which result in the lysosomal accumulation of GAGs. Similar to glycogenoses, these lysosomal storage diseases are characterised by an accumulation of autophagosomes as a result of defective autophagosome-lysosome fusion [178]. *In vitro* mouse models of MSD, with knock-out of the sulphatase modifying factor 1 (*Sumf1*) have identified that its knock-out leads to an accumulation of cholesterol in lysosomal membranes which perturbs SNARE proteins and thus fusion capabilities of the lysosomes [179]. Specific knock-out of *sumf1* in astrocytes can cause neurodegenerative phenotypes [180], in osteoclasts causes reduced cell survival [181] and reduced mitophagy due to

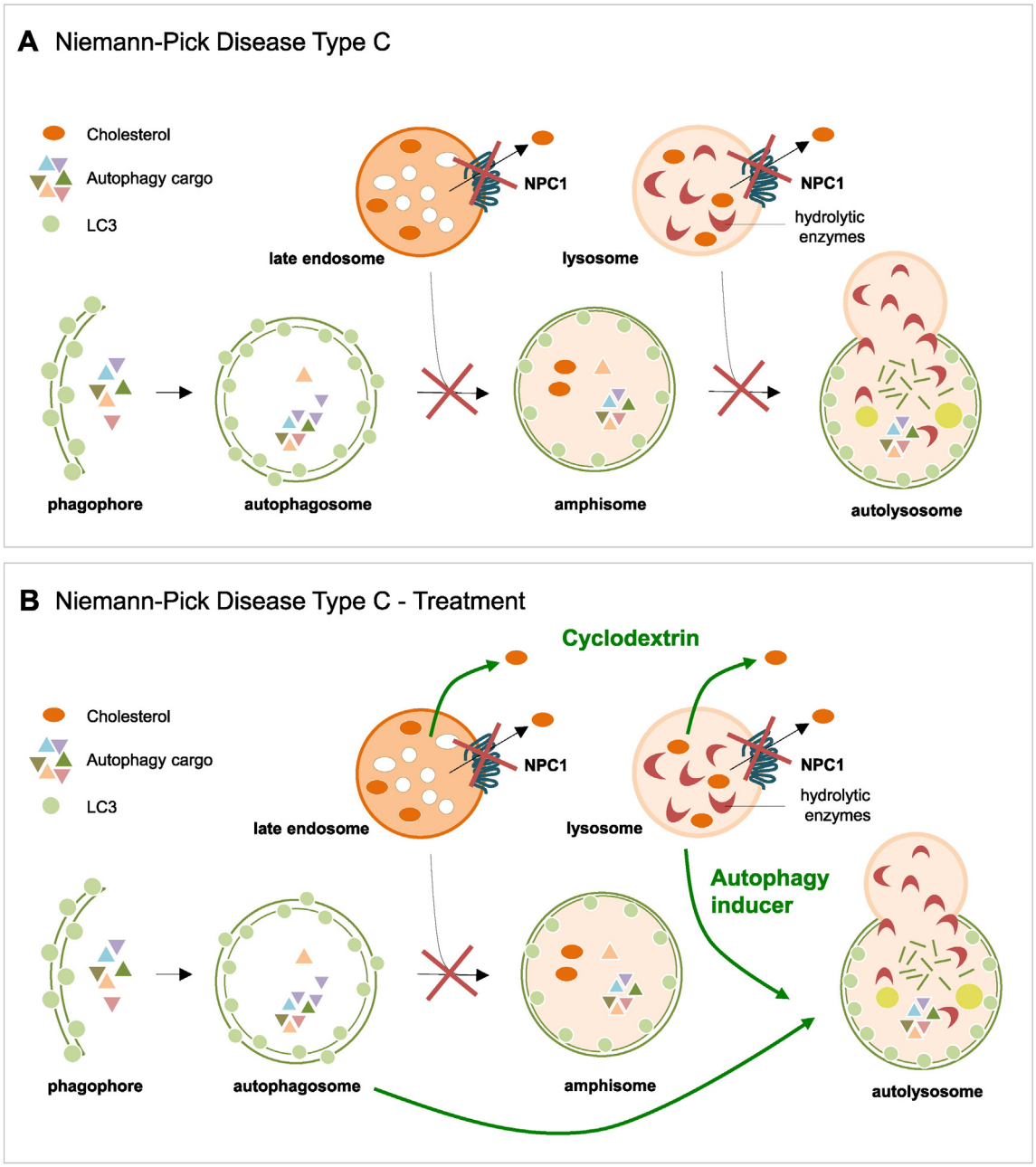

Fig. 3. Defective autophagy in Niemann–Pick Disease Type C as a target for therapeutic intervention: A — the disease is characterised by a block in autophagic flux arising from the impaired formation of amphisomes, in turn caused by a failure in the SNARE machinery that is required for the fusion between autophagosomes and late endosomes. B — a combination of cholesterol releasing agent cyclodextrin with autophagy-inducing drugs has been proposed as a potential therapeutic intervention (see text for further details). The bold green arrows represent the mechanisms allowing to overcome the defects in autolysosomal pathway in Niemann–Pick Disease Type C by inducers of autophagy (e.g. rapamycin) and cyclodextrin.

reduced levels of parkin [182]. Modifying autophagy potential within these models is an attractive therapeutic option that requires further investigation.

6.6. Niemann–Pick disease

Niemann–Pick disease is a group of inherited, metabolic, lipid/lysosomal storage disorders comprised of Niemann–Pick types A (NPA), B (NPB) and C (NPC) disease. NPA and NPB disease are caused by mutations in *SMPD1* gene encoding sphingomyelin phosphodiesterase 1, which leads to insufficient activity of the enzyme acid sphingomyelinase (ASM) and an accumulation of sphingomyelin [183,184]. NPC disease is caused by the mutations in *NPC1* or *NPC2* gene encoding proteins essential for cholesterol efflux from the late endosomal/lysosomal compartments and leads to a build-up of cholesterol in these compartments [185–187]. NPC1 is the most common form in this class of diseases that primarily affects children. NPA is primarily a severe neurologic disease-causing brain damage whereas NPB is associated with enlarged liver and spleen (hepatosplenomegaly) and respiratory problems [188]. NPC on the other hand is associated with hepatomegaly, splenomegaly, psychomotor retardation and neurodegeneration along with other neurological symptoms [189].

Studies in NPC1 patient fibroblasts, NPC knock-out iPSC and *Npc1*$^{-/-}$ mouse models have highlighted a potential role for autophagy in NPC1 disease. Specifically, accumulation of autophagosomes/LC3-II, autophagic multivesicular structures, lysosomes and cathepsin D has been observed in the brain and neuronal cultures of NPC1 mutant mice (*Npc1*$^{-/-}$), NPC1 patient fibroblasts, and in human embryonic

stem cell (hESC)-derived neurons with NPC1 knockdown [134,190–194]. Although some of these studies have implied the accumulation of autophagosomes as a result of autophagy induction, later studies have defined this phenotype to be caused by a block in autophagic flux. We have recently shown that the defect in autophagy in cells from *Npc1*$^{-/-}$ mice is caused by the impaired formation of amphisomes which results from a failure in the SNARE machinery required for the fusion of autophagosomes and late endosomes [161] (Fig. 3). We further confirmed dysfunctional autophagic flux in NPC1 patient-specific iPSC-derived neuronal and hepatic cells, which are the two primary disease-affected cell types [161]. A separate study has also demonstrated that accumulation of lysosomal cholesterol causes aberrant sequestration of SNARE proteins and disrupts the fusion events between autophagosomes with late endosomes and lysosomes [179] (Fig. 3). Consequently, the clearance of autophagic cargo is diminished as evident from the build-up of p62 and damaged mitochondria [194–196]. A recent study has suggested another potential mechanism that may underlie the impairment in autophagy observed in NPC1 which is linked to reduced sphingosine kinase activity and lowered levels of vascular endothelial growth factor (VEGF). The result is an inhibition of autophagosome maturation via abnormal sphingosine accumulation [197]. These defects in autophagic flux have been suggested to cause cellular toxicity in NPC1 mouse and iPSC models [161,193,195,197,198]. In addition, lipids such as cholesterol are cleared through autophagy as evident by its elevated levels in autophagy-deficient (*Atg5*$^{-/-}$) cells that recapitulates NPC1 cellular phenotype [80]. Our data imply that defective autophagy arising due to *NPC1* mutations is likely to act as a positive feedback loop in increasing cholesterol load, thus augmenting the disease phenotype [196,199].

Studies on perturbations in autophagy as an underlying factor in NPA and NPB disease pathology are limited. A recent study has shown accumulation of sphingomyelin and autophagosomes in neurons from *ASM* knockout mice and NPA patient fibroblasts, an effect that can be partially reversed by inhibition of sphingolipid synthesis with Fumonisin B1. The defect in autophagy was attributed to improper clearance of autolysosomes due to sphingomyelin-induced lysosomal membrane permeabilisation that leads to cytosolic release of its \proteases [200]. Age-dependent retinal degeneration was also found in *ASM* knockout mice which was associated with a build-up of autophagosomes, possibility implicating a role of defective autophagy in the degenerative process [201].

A number of therapeutic strategies have been shown for NPC1 disease. Miglustat, an inhibitor of glycosphingolipid synthesis has been approved for the treatment of NPC disease as a substrate reduction approach and it has been shown to delay disease progression in transgenic mice [202,203]. Other avenues reported are the use of the neurosteroid, allopregnanolone and replenishment of VEGF that were beneficial in NPC1 mouse and iPSC models, respectively [191,197]. Hydroxypropyl-β-cyclodextrin (HPβCD), which promotes cholesterol-release from lysosomal compartments, has also been identified as a therapeutic candidate to treat NPC1 disease [194,204–207] (Fig. 3). A small scale trial of treatment with HPβCD showed phenotypic improvement, however there were issues with drug delivery though the blood brain barrier [208]. Although treatment with HPβCD lowered cholesterol accumulation, it had some adverse side effects in animals [209]. Moreover, high doses of this compound had a negative impact on autophagic flux and neurotoxic effects that could exacerbate the disease phenotype [195,196,210]. On the other hand, we showed that stimulating autophagy could bypass the autophagic block at the amphisomes stage by causing autophagosomes to directly fuse with the lysosomes, thus restoring autophagic flux and enabling the clearance of accumulated autophagic cargo. Although, of note, restoration of autophagic flux had negligible effect on lysosomal cholesterol. We found that stimulating autophagy with rapamycin (mTOR inhibitor) or carbamazepine (an inositol-lowering agent and mTOR-independent autophagy inducer) rescued the autophagy defects and improved cell viability in NPC1 iPSC-derived neuronal and hepatic cells; however, certain autophagy-inducing compounds such as trehalose, verapamil and BRD5631 were effective only in neurons [195,196,211]. Although a study has indicated impaired lysosomal proteolysis in NPC1 patient fibroblasts [212], our data and other reports imply that the functionality of lysosomes and cathepsin activity are not compromised [190,191,195,196]. We have proposed a combination treatment strategy using lower doses of HPβCD (that partially reduce cholesterol without perturbing autophagic flux) coupled with autophagy stimulators (for restoring autophagic flux) to abrogate the abnormal cholesterol and autophagy phenotypes [195,196,199] (Fig. 3). Interestingly, a recent study has developed polymeric supermolecules designed to deliver prodrugs into cells called β-cyclodextrin-threaded biocleavable polyrotaxanes [213,214]. These were able to reduce both the cholesterol and autophagy defects in NPC1 patient fibroblasts [215]. In future, it would be interesting to assess the protective effects of this compound or autophagy enhancers and combinatorial treatments in NPC1 models *in vivo*.

6.7. Mucolipidosis type IV

Mucolipidosis type IV (MLIV) is a neurodegenerative lipid/lysosomal storage disorder; the most common symptoms include ocular aberrations, progressive mental defects and motor deterioration [216–218]. MLIV is caused by mutations in the *MCOLN1* gene, which encodes a transient receptor potential cation channel called mucolipin-1 (TRPML1) which is involved in calcium signalling and transport [219–221].

A number of studies have implicated a role for MCOLN1 in lysosomal acidification and secretion, autophagosome maturation and mitochondrial turnover [222–225]. Thus, disease-causing mutations in *MCOLN1* are likely to impact on the autophagy pathway. Indeed, MLIV patient fibroblasts exhibit an accumulation of autophagosomes and p62, suggesting there is a block in autophagic flux [225]. Likewise, impairment in autophagic flux associated with accumulation of LC3-II, LAMP1, p62, polyubiquitinated proteins and membranous intracytoplasmic storage bodies was seen in MCOLN1-deficient mouse neurons generated from the cerebrum of the *Mcoln1*$^{-/-}$ embryos [226]. Consequently, defective mitochondrial recycling through the autophagy pathway and increased mitochondrial fragmentation has been shown in MLIV patient fibroblasts [223]. Over-acidified lysosomes were also observed in MLIV patient fibroblasts that led to the malfunction of acidic lipase activity and lipid hydrolysis, which could be rescued by treatment with nigericin (an H+/K+ exchange ionophore) or chloroquine (accumulates in acidic spaces and dissipates low pH) [222]. A *Drosophila* model of MLIV, which exhibited key disease phenotypes such as intracellular accumulation of macromolecules, motor defects, and neurodegeneration, was characterised by defective autophagy that resulted in oxidative stress and improper clearance of apoptotic cells [227]. Moreover, impairment in CMA has been reported in MLIV patient fibroblasts wherein TRPML1 interacts with the chaperone proteins, Hsc70 and Hsc40 [228]. However, further studies will be of interest to understand the mechanistic details of how this protein regulates CMA, and how deregulation of this process contributes to the disease pathogenesis.

There are currently no treatments for MLIV although a small molecule, MK6-83 has been recently identified that was able to restore the function of the defective TRPML1 channel and rescue the disease-associated abnormalities in MLIV patient fibroblasts with specific point mutations [229]. While this provides a promising avenue for treatment, the therapeutic effect of autophagy modulation by small molecules is currently unknown.

6.8. Neuronal ceroid lipofuscinosis

Neuronal ceroid lipofuscinosis (NCL) is a family of genetically distinct neurodegenerative, lysosomal storage disorders affecting young children [230]. Symptoms include impaired vision, seizures, mental

retardation, dementia, motor deterioration and muscle twitching [231,232]. The older classification of NCL is based upon the age of onset, such as early infantile (Santavuori-Haltia disease), late infantile (Jansky–Bielschowsky disease), juvenile (Batten disease) and adult (Kufs disease) forms, whereas the newer classification is divided by the 13 associated genes identified so far [161,233].

NCL exhibits abnormal accumulation of lipofuscin, which are lipopigments made up of fats and proteins, in neuronal cells and other tissues [234]. Initial studies have shown an accumulation of lysosomal ceroid lipofuscin and autophagosomes in the neurons of mice deficient for lysosomal proteases, cathepsin D or cathepsins B and L, implicating that cathepsin-deficient mouse could be used for studying the pathogenesis of NCL [235,236]. A subsequent study has shown that mice deficient for both cathepsin D and Bax (a pro-apoptotic protein) displayed defective autophagy and a neurodegenerative phenotype, with the absence of caspase 3 activation, suggesting that neuronal cell death may be caused by genetic disruption of lysosomal function and possibly by autophagy dysfunction [237]. In addition, disruption of autophagy associated with defective autophagosome maturation was found in a knock-in mouse model of Batten disease (*Cln3*$^{\Delta ex7/8}$ mice), the most common form of NCL (NCL3) which is caused by mutations in *CLN3* that encodes an endosomal/lysosomal membrane protein called CLN3 or battenin [238,239]. Likewise, accumulation of mitochondrial ATPase subunit C protein in autophagosomal/lysosomal compartments in NCL3 mouse and human iPSC models suggests defective mitochondrial turnover through the autophagy pathway [236,239–241]. An elevation in the levels of α-synuclein oligomers and gangliosides GM1, GM2, and GM3 was also found in NCL3 patient lymphoblast cells [242]. Furthermore, age-dependent elevation in LC3-II, p62 aggregates and ubiquitinated proteins were found in the brain of NCL6 mouse model (Cln6-defective *nclf* mice) [243], as well as accumulation of autophagic vacuoles seen in NCL6 patient fibroblasts [244]. A chemical screen in a cellular model of NCL3 (*Cln3*$^{\Delta ex7/8}$), stably expressing GFP-LC3 has identified that the autophagy blocker, thapsigargin causes enhanced Ca^{2+} sensitivity that may lead to aberrant Ca^{2+} signalling and vesicular trafficking defects [245]. Moreover, impairment in actin-dependent processes through deregulated ARF1-Cdc42 pathway in *CLN3* mutant cells may contribute to defective vesicular trafficking events including autophagy [246].

There is currently no cure for NCL. Treatment of the disease is based around alleviating the symptoms caused by the neurodegeneration. However, gene delivery through adenoviral vectors to replace the deficient *CLN2* gene was successfully demonstrated in rats and non-human primates [247]. Due to the success of these animal trials, a small scale clinical trial showed a small but non-significant improvement [248]. Clearly there are safety issues with using viruses as gene delivery targets. Interestingly, autophagy enhancers such as lithium and L-690,330 (inositol monophosphatase inhibitors) [67] were shown to reduce the abnormal accumulation of autophagosomes, mitochondrial ATP synthase subunit C and lipofuscin in *Cln3* mutant knock-in cerebellar cells [161]. This study points to the need for a deeper understanding of the mechanisms of defective autophagy and its therapeutic application in NCL.

7. Conclusions

While in recent years an important role of autophagy in the maintenance of lipo-homeostasis has been revealed, the contribution of autophagy pathways to the pathology in many lipid storage disorders still remains poorly understood. Further work will be required to better define the mechanistic details of autophagy perturbations in each specific disease and their contribution to the pathology. These studies will undoubtedly inform new treatment strategies as has already been demonstrated in case of several lipid storage disorders.

Conflict of Interest

Authors declare no conflict of interest.

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